AMENDMENTS TO THE SPECIFICATION:

Please replace the following paragraphs:

Paragraph at page 1, lines 1:

TITLE

~~PLANT PROTEINASES~~ Polynucleotides Encoding Plant Cysteine Proteases

This application is a CIP of U.S. Application No. 09/501,423, filed February 9, 2000, which claims the benefit of U.S. Provisional Application No. 60/119,599, filed February 10, 1999, whose contents are hereby incorporated by reference.

Paragraph at page39, line 1:

TITLE

~~PLANT PROTEINASES~~ Polynucleotides Encoding Plant Cysteine Proteases

ABSTRACT OF THE DISCLOSURE

This invention relates to an isolated nucleic acid fragment encoding a proteinase. The invention also relates to the construction of a chimeric gene encoding all or a portion of the proteinase, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the proteinase in a transformed host cell.

Paragraph at page2, lines 19:

The present invention relates to isolated polynucleotides comprising a nucleotide sequence encoding a polypeptide of at least 150 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of a cysteine protease [[1]] polypeptide of SEQ ID NOs:14, 16, 18, and 20. The present invention also relates to an isolated polynucleotide comprising the complement of the nucleotide sequences described above.

Paragraph at page2, lines 25-30:

The present invention relates to isolated polynucleotides comprising a nucleotide sequence encoding a polypeptide of at least 200 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of a cysteine protease [[2]] polypeptide of SEQ ID NOs:22 and 24. The present invention also relates to an isolated polynucleotide comprising the complement of the nucleotide sequences described above.

Paragraph at page 3, lines 28-37:

The present invention relates to a cysteine protease [[1]] polypeptide of at least 150 amino acids comprising at least 95% homology based on the Clustal method of alignment compared to a polypeptide selected from the group consisting of SEQ ID NOs:8, 10, 36, and 38.

The present invention relates to a cysteine protease [[2]] polypeptide of at least 200 amino acids comprising at least 80% homology based on the Clustal method of alignment compared to a polypeptide selected from the group consisting of SEQ ID NOs:12 and 40.

Table 1 at page 5-6:

TABLE 1

PLANT PROTEINASES

Protein	Clone Designation	SEQ ID NO: (Nucleotide)	SEQ ID NO: (Amino Acid)
Corn Calpain p94 Subunit	cbn2.pk0039.c2	1	2
Rice Calpain p94 Subunit	rsl1n.pk013.h14	3	4
Soybean Calpain p94 Subunit	ses9c.pk001.j23	5	6
Rice Cysteine Protease [[1]]	rr1.pk084.j16	13	14
Wheat Cysteine Protease [[1]]	Contig of: wdk1c.pk009.j19 wre1n.pk164.b11	15	16
Soybean Cysteine Protease [[2]]	Contig of: sgs2c.pk002.p14 srr3c.pk003.d10 scb1c.pk003.d8	21	22
Corn CLP ATP Binding Subunit	p0110.cgsmk69r	25	26
Rice CLP ATP Binding Subunit	Contig of: rlr6.pk0083.f9 rlr24.pk0088.f7 rlr6.pk0029.d7	33	34
Wheat CLP ATP Binding Subunit	wlm96.pk032.n8	29	30
Corn CLP Proteolytic Subunit	p0060.coran66r	37	38
Rice CLP Proteolytic Subunit	rsr9n.pk004.p5	39	40
Soybean CLP Proteolytic Subunit	scb1c.pk004.k24	41	42
Wheat CLP Proteolytic Subunit	wle1n.pk0042.f7	43	44
Wheat CLP Proteolytic Subunit	wlk8.pk0006.a4	45	46
Corn Calpain p94 Subunit	cbn2.pk0039.c2:fis	7	8

Rice Calpain p94 Subunit	rsl1n.pk013.h14:fis	9	10
Soybean Calpain p94 Subunit	ses9c.pk001.j23:fis	11	12
Rice Cysteine Protease [[1]]	rr1.pk084.j16:fis	17	18
Wheat Cysteine Protease [[1]]	wdk1c.pk009.j19:fis	19	20
Soybean Cysteine Protease [[2]]	srr3c.pk003.d10:fis	23	24
Corn CLP ATP Binding Subunit	p0110.cgsmk69r:fis	31	32
Rice CLP ATP Binding Subunit	rlr24.pk0088.f7:fis	27	28
Wheat CLP ATP Binding Subunit	wlm96.pk032.n8:fis	35	36
Corn CLP Proteolytic Subunit	p0060.coran66r:fis	47	48
Rice CLP Proteolytic Subunit	rsr9n.pk004.p5:fis	49	50
Soybean CLP Proteolytic Subunit	scb1c.pk004.k24:fis	51	52
Wheat CLP Proteolytic Subunit	wle1n.pk0042.f7:fis	53	54
Wheat CLP Proteolytic Subunit	wlk8.pk0006.a4:fis	55	56

Paragraph at page 14, lines 3-15:

For example, genes encoding other cysteine proteases 1s, ~~cysteine protase 2s,~~ calpain p94s, CLPAs, or CLPPs, either as cDNAs or genomic DNAs, could be isolated directly by using all or a portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired plant employing methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis). Moreover, the entire sequences can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primer DNA labeling, nick translation, or end-labeling techniques, or RNA probes using available *in vitro* transcription systems. In addition, specific primers can be designed and used to amplify a part or all of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

Paragraph at page 14, line 16 through page 15 line 12:

In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the instant nucleic acid fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding plant genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al. (1988) *Proc. Natl. Acad. Sci. USA 85*:8998-9002) to generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems (BRL), specific 3' or 5' cDNA fragments can be isolated (Ohara et al. (1989) *Proc. Natl. Acad. Sci. USA 86*:5673-5677; Loh et al. (1989) *Science 243*:217-220). Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman and Martin (1989) *Techniques 1*:165). Consequently, a polynucleotide comprising a nucleotide sequence of at least one of 60 (preferably one of at least 40, most preferably one of at least 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9,11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, and 55 and the complement of such nucleotide sequences may be used in such methods to obtain a nucleic acid fragment encoding a substantial portion of an amino acid sequence of a polypeptide. The present invention relates to a method of obtaining a nucleic acid fragment encoding a substantial portion of a proteinase polypeptide (such as a cysteine protease ~~1, a cysteine protase 2~~, a calpain large subunit, a CLP protease proteolytic subunit or a CLP protease ATP binding subunit) preferably a substantial portion of a plant polypeptide of a gene, comprising the steps of : synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of 60 (preferably at least one of 40, most preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9,11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, and 55, and the complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a portion of a cysteine protease ~~I, a cysteine protase 2~~, a

calpain large subunit, a CLP protease proteolytic subunit or a CLP protease ATP binding subunit polypeptide.

Paragraph at page 23, line 11 through page 26 line 6:

EXAMPLE 4

Characterization of cDNA Clones Encoding Cysteine Protease ~~[1]~~

The BLASTX search using the EST sequences from clones listed in Table 3 revealed similarity of the polypeptides encoded by the cDNAs to cysteine protease [[1]] from *Zea mays* (NCBI General Identifier No. 1706260). Shown in Table 6 are the BLAST results for individual ESTs ("EST"), or for contigs assembled from two or more ESTs ("Contig"):

TABLE 6

BLAST Results for Sequences Encoding Polypeptides Homologous to Cysteine Protease [[1]]

Clone	Status	BLAST pLog Score 1706260
rr1.pk084.j16	EST	94.52
Contig of: wdk1c.pk009.j19 wre1n.pk164.b11	Contig	130.00

The entire cDNA insert in clones rr1.pk084.j16 and wdk1c.pk009.j19 was determined. The BLASTP search using the amino acid sequences from clones listed in Table 7 revealed similarity of the polypeptides encoded by the cDNAs to cysteine protease [[1]] from *Zea mays* (NCBI General Identifier No. 1706260). Shown in Table 7 are the BLAST results for the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"):

TABLE 7

BLAST Results for Sequences Encoding Polypeptides Homologous to Cysteine Protease [[1]]

Clone	Status	BLAST pLog Score 1706260
rr1.pk084.j16:fis	FIS	158.00
wdk1c.pk009.j19:fis	FIS	110.00

Amino acid sequence alignments using the Clustal method of alignment indicates that the rice sequence starts 88 amino acids down stream from the corn starting methionine, and that the wheat sequence starts 163 amino acids down stream from the corn starting methionine. The corn sequence has a signal sequence (amino acids 1-19) and a mature protein which corresponds to amino acids 137 through 371. Thus, the rice and wheat sequences included here contain the entire mature protein. The data in Table 8 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:14, 16, 18, and 20 and the *Zea mays* sequence (NCBI General Identifier No. 1706260).

TABLE 8

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to Cysteine Protease 1

SEQ ID NO.	Percent Identity to 1706260
14	88.0
16	87.1
18	90.9
20	86.3

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS. 5*:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments and BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode a substantial portion of a rice and a wheat cysteine protease [[1]]. These sequences represent the first rice and wheat sequences encoding cysteine protease [[1]].

EXAMPLE 5

Characterization of cDNA Clones Encoding Cysteine Protease [[2]]

The BLASTX search using the EST sequences from clones listed in Table 9 revealed similarity of the polypeptides encoded by the cDNAs to cysteine protease [[2]] from *Phaseolus vulgaris* (NCBI General Identifier No. 2511691). Shown in Table 9 are the BLAST results for sequences of contigs assembled from two or more ESTs ("Contig"):

TABLE 9

BLAST Results for Sequences Encoding Polypeptides Homologous to Cysteine Protease [[2]]

Clone	Status	BLAST pLog Score 2511691
Contig of: sgs2c.pk002.p14 srr3c.pk003.d10 scb1c.pk003.d8	Contig	97.70

The sequence of the entire cDNA insert in clone srr3c.pk003.d10 was determined. The BLASTP search using the amino acid sequences from clones listed in Table 10 revealed similarity of the polypeptides encoded by the cDNAs to cysteine protease [[2]] from *Phaseolus vulgaris* (NCBI General Identifier No. 2511691). Shown in Table 10 are the BLAST results for the sequences of the entire cDNA inserts comprising the indicated cDNA clones encoding the entire protein ("CGS"):

TABLE 10

BLAST Results for Sequences Encoding Polypeptides Homologous to Cysteine Protease [[2]]

Clone	Status	BLAST pLog Score 2511691
srr3c.pk003.d10:fis	CGS	169.00

The data in Table 11 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:22 and 24 and the *Phaseolus vulgaris* sequence (NCBI General Identifier No. 2511691).

TABLE 11

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to Cysteine Protease [[2]]

SEQ ID NO.	Percent Identity to 2511691
22	67.9
24	75.1
12	67.9
40	75.1

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS. 5*:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments and BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode a substantial portion and an entire soybean cysteine protease [[2]]. These sequences represent the first soybean sequences encoding cysteine protease [[2]].